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Knowledge, attitudes and practices of caregivers towards inhaler use in asthmatic children

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Abstract

Aim: This study aimed to assess the knowledge, attitudes, and practices (KAPs) of caregivers towards asthma disease, especially in using prescribed inhalers for children with asthma at Hue Central Hospital, Vietnam.

Material and Methods: This was a cross sectional survey based study conducted at the Pediatric Pulmonary Department of Hue Central Hospital. A questionnaire that assessed KAPs regarding inhalers used in asthma was distributed to 278 parents of children who had asthma.

Results: Only 5.0% of the participants correctly answered > 75% of the questions assessing knowledge, 10.8% gave positive responses to > 75% of the questions related to attitudes, whereas 48.6% correctly performed of practices of nebulizer and 8.6% correctly performed of practices of MDI (metered-dose inhaler) with chamber. There was a relationship between knowledge and attitudes toward the use of inhaler ($p<0.05$). The proportion of caregivers having good practices of nebulizers was higher in the group with good asthmatic knowledge than in the other groups (70.2% compared to 50.4% and 35.2%, $p < 0.05$), in the group with good asthma practices compared to the other groups (20.8% vs 6.7% and 4.7%, $p < 0.05$). Good practice of MDI with spacer was found to be better in the group of caregivers of children with a history of asthma than in the group without a history of asthma (13.8% vs. 3.6%, $p < 0.05$).

Discussion: Although a minority of the caregivers had a good level of knowledge and attitudes, they tended to demonstrate good practices with regard to the use of an inhaler, especially using nebulizer.

Keywords

Knowledge, Attitudes, Practices, Inhaler Use, Childhood Asthma

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Introduction

Asthma is one of the most common chronic diseases in the world. The World Health Organization (WHO) estimates that there are currently about 300 million people with asthma worldwide, and with the current increasing trend, it is expected that this number will reach 400 million people with asthma by 2025 [1]. In children, asthma is the most common chronic disease, ranking among the top 20 diseases in disease-adjusted life years in children worldwide [2]. According to the International Study of Asthma and Allergy in Children (ISAAC) from phase I to phase III, the global rate of asthma symptoms in children and adolescents ranged from 11.1-11.6% and from 13.2-13.7% respectively [3]. World data records showed that the death rate due to asthma in children ranged from 0 - 0.7/100.000 people [4]. Many surveys still show that the current level of asthma control in many countries is still below optimal levels, especially in developing countries, increasing the burden of asthma [5, 6]. It is worth mentioning that the appropriate treatment of pediatric asthma patients does not solely rely on accurate evaluation and prompt intervention by healthcare professionals. It is also greatly affected by parental/caregivers' knowledge, attitudes, and practices (KAPs) regarding the use of inhalers in asthmatic children. Only a few studies have assessed this issue among children with asthma worldwide. The aim of this study was to evaluate the factors affecting knowledge, attitudes and practices of parents/caregivers towards inhaler use in asthmatic children.

Material and Methods

Patients

The study subjects were the parents and direct caregivers of pediatric patients with asthma aged between 1 and 15 years admitted to the Respiratory Department, Pediatric Center, Hue Central Hospital. 285 parents/caregivers were invited to participate in this survey during the study period.

Method

A cross-sectional non-interventional survey-based study was conducted at the Respiratory Department, Pediatric Center, Hue Central Hospital between January 2022 and December 2023.

Data collection methods

We developed a questionnaire to assess caregiver KAPS regarding the use of inhalers [Appendix 1]. Knowledge of parents/caregivers regarding reliever and controller medications consists of 22 items, and each correct answer is given a score of 1. A score of ≤ 50% total points was considered as poor, 51-75% total points as fair and > 75% total points as good. Attitudes of parents/caregivers regarding reliever and controller medications consist of 17 items. Each correct answer is given a score of 1. A score of ≤ 50% total points was considered as negative, 51-75% total points as neutral and > 75% total points as positive [7]. The practice of parents/caregivers using nebulizer consists of 8 items. Good practice would be defined if parents/caregivers performed successfully the two critical steps 3 and 4 (*), while other steps might be partially accomplished; not good practice if parents/caregivers did not perform successfully the two critical steps (*), while other steps might be partially accomplished [8]. The practice of parents/caregivers using a metered-dose inhaler (MDI) with spacer consists of 5 items. Good practice would be defined if parents/caregivers correctly performed all critical steps (*); and not good practice if parents/caregivers performed incorrectly one or more critical steps (*) [9-11].

Procedure

Caregivers were notified about the purpose of the study and they were invited voluntarily. After providing written informed consent, they completed the questionnaire through a face-to-face interview with medical doctors. Each child will have only one caregiver who will be interviewed. The inhaler technique was demonstrated by caregivers by face-to-face demonstration.

Statistical analysis

Data were analyzed according to knowledge, attitude and practices about the asthmatic disease and towards inhaler use. All statistical analysis was performed using SPSS v.18.0 (IBM Corp, Armonk, NY).

Ethical approval

This study was approved by the Hue University Ethics Committee (Date: 2022-10-10, No: 22/NCKH-DHH). Consent was obtained from all participants in this study.

Results

Knowledge

General knowledge about asthma medications: most caregivers did not have good knowledge, accounting for 14 (5.0%), the percentage of having fair and poor knowledge were 11.5% and 83.5% respectively. Regarding using reliever asthma medication knowledge, the percentage of having good, fair and poor knowledge accounted for 5.0%, 11.9% and 83.1% respectively. The percentage of having good, fair and poor knowledge of using controller asthma medication were 4.7%, 8.6% and 86.7% respectively.

Attitudes

The percentage of caregivers who had a positive attitude toward the use of asthma medication was 10.8%, whereas 28.1% and 61.2% of the caregivers exhibited fair and negative attitudes, respectively. Regarding the attitude toward the use of reliever asthma medication, the percentage of caregivers who had a positive, fair and poor attitude were 9.4%, 28.1% and 62.6%. These percentages were similar for the caregivers who toward the use of controller asthma medication, with 12.6% caregivers having a positive attitude, 18.7% of them having fair attitudes and the majority of them (68.7%) having negative attitudes.

Practices

The percentage of caregivers who have good practices in the use of nebulizer was 48.6%, whereas 51.4% had bad practices. Regarding practices in the use of MDIs with spacers, there were only 8.6% of caregivers who had good practices and 91.4% of caregivers who had bad practices.

Factors affecting caregivers' knowledge towards reliever and controller medications

Caregivers' knowledge about asthma medication was not significantly associated with caregivers' gender, age, job, education, asthma history of patients and their families, severity of flare-ups, and severity of asthma ($p > 0.05$) (Table 1).

The proportion of caregivers with good knowledge about medications was significantly lower in the group that received

asthma information from relatives compared to the group that did not receive those (0.0% vs. 7.7%, p < 0.05). However, this rate was not correlated with self-searching information on the internet, radio or receiving information from healthcare staff. The percentage of caregivers with good knowledge about medications was higher in the group with good asthma knowledge than in the other groups (15.8% vs 3.8% and 0.0%, p < 0.05); in the group with good asthma attitudes than the other groups (12.5% vs 0.7% and 0.0%, p < 0.05); in the group with good asthma practices than the other groups (9.6% vs 1.1% and 1.6%, p < 0.05) (Table 1).

Factors affecting caregivers' attitudes towards reliever and controller medications

Caregiver's attitudes about asthma medications were not significantly associated with caregivers' gender, job, asthma history of patients and their families, severity of flare-ups, and severity of asthma (p > 0.05); but significantly associated with caregivers' age and education (p < 0.05) (Table 2).

The proportion of caregivers with good attitudes about medications was significantly lower in the group that received asthma information from relatives compared to the group that did not receive those (8.2% vs. 12.2%, p < 0.05), However, this rate was not correlated with self-searching information on the internet, radio or receiving information from healthcare staff. The proportion of caregivers with good attitudes about medications was significantly higher in the group with good asthma knowledge than in the other groups (24.6% vs 11.5% and 1.1%, p < 0.05); in the group with good asthma attitudes

Table 1. Factors affecting caregiver's knowledge toward reliever and controller asthma medications

		Knowledge						p
		Good		Fair		Poor		
		n	%	n	%	n	%	
Gender of caregiver	Male	0	0	2	9.5	19	90.5	>0.05
	Female	14	5.4	30	11.7	213	82.9	
Age of caregiver	<30	6	5.1	14	12.0	97	82.9	>0.05
	31 - 40	7	5.5	17	13.4	103	81.1	
	>40	1	2.9	1	2.9	32	94.1	
Education level of caregiver	Under high school	2	2.9	5	7.4	61	89.7	>0.05
	High school	5	3.9	13	10.2	109	85.8	
	University and above	7	8.4	14	16.9	62	74.7	
Job of caregiver	Officer	6	7.8	16	20.8	55	71.4	>0.05
	Worker	0	0	2	4.8	40	95.2	
	Farmer	0	0	0	0	13	100	
	Small business	3	5.1	4	6.8	52	88.1	
	Housewife	4	7.7	5	9.6	43	82.7	
	Other	1	2.9	5	14.3	29	82.9	
Receive asthma information from healthcare	Yes	13	6.3	23	11.2	169	82.4	>0.05
	No	1	1.4	9	12.3	63	86.3	
Search asthma information from books and newspapers	Yes	5	5.7	5	5.7	78	88.6	>0.05
	No	9	4.7	27	14.2	154	81.1	
Receive asthma information from relative	Yes	0	0	9	9.3	88	90.7	<0.05
	No	14	7.7	23	12.7	144	79.6	
Asthma history	Yes	10	7.2	18	13.0	110	79.7	>0.05
	No	4	2.9	14	10.0	122	87.1	
Asthma family history	Yes	4	3.8	17	16.0	85	80.2	>0.05
	No	10	5.8	15	8.7	147	85.5	
Severity of acute asthma attack	Mild	0	0	0	0	4	100	>0.05
	Moderate	13	6.2	23	10.9	175	82.9	
	Severe	1	1.6	9	14.3	53	84.1	
Severity of asthma	Intermittent	11	5.2	25	11.7	177	83.1	>0.05
	Mild persistent	2	4.2	6	12.5	40	83.3	
	Moderate persistent	1	5.9	1	5.9	15	88.2	
Caregiver's knowledge	Good	9	15.8	14	24.6	34	59.6	<0.05
	Fair	5	3.8	15	11.5	111	84.7	
	Poor	0	0	3	3.3	87	96.7	
Caregiver's attitudes	Positive	13	12.5	15	14.4	76	73.1	<0.05
	Neutral	1	0.7	16	11.9	117	87.3	
	Negative	0	0	1	2.5	39	97.5	
Caregiver's practices	Good	12	9.6	29	23.2	84	67.2	<0.05
	Fair	1	1.1	2	2.2	86	96.6	
	Bad	1	1.6	1	1.6	62	96.9	

than the other groups (20.2%% vs 6.7% and 0.0%, p < 0.05); in the group with good asthma practices than the other groups (20.8% vs 3.4% and 1.6%, p < 0.05) (Table 2).

Factors affecting caregivers' practices of using nebulizers and MDIs with spacers

Caregivers' practices of using nebulizers were not significantly associated with caregivers' gender, age, job and education, asthma history of patients and their families, severity of flare-ups, and severity of asthma (p > 0.05). However, there was a significant relationship between caregivers' knowledge and attitudes about reliever medications, caregivers' attitudes about controller medications and their practice of using nebulizers (p < 0.05) (Table 3).

The proportion of caregivers with good practices of using nebulizer was significantly lower in the group that received asthma information from relatives compared to the group that did not receive those (35.1% vs. 55.8%, p < 0.05). However, this rate was not correlated with self-searching information on the internet, radio or receiving information from healthcare staff. And the proportion of caregivers with good practices of using nebulizer was significantly higher in the group with good asthma knowledge than in the other groups (70.2% vs 50.4% and 35.2%, p < 0.05); in the group with good asthma practices than the other groups (20.8% vs 6.7% and 4.7%, p < 0.05) (Table 3).

Caregivers' practices of using MDIs with spacers were not

Table 2. Factors affecting caregiver's attitudes toward reliever and controller asthma medications

		Attitudes						p
		Positive		Fair		Negative		
		n	%	n	%	n	%	
Gender of caregiver	Male	2	9.5	2	9.5	17	81.0	>0.05
	Female	28	10.9	76	29.6	153	59.5	
Age of caregiver	<30	11	9.4	42	35.9	64	54.7	<0.05
	31 - 40	15	11.8	35	27.6	77	60.6	
	>40	4	11.8	1	2.9	29	85.3	
Education of caregiver	Under high school	6	8.8	12	17.6	50	73.5	<0.05
	High school	10	7.9	39	30.7	78	61.4	
	University and above	14	16.9	27	32.5	42	50.6	
Job of caregiver	Officer	12	15.6	24	31.2	41	53.2	>0.05
	Worker	3	7.1	12	28.6	27	64.3	
	Farmer	0	0	2	15.4	11	84.6	
	Small business	6	10.2	16	27.1	37	62.7	
	Housewife	5	9.6	15	28.8	32	61.5	
	Other	4	11.4	9	25.7	22	62.9	
Receive asthma information from healthcare	Yes	24	11.7	58	28.3	123	60.0	>0.05
	No	6	8.2	20	27.4	47	64.4	
Search asthma information from books and newspapers	Yes	10	11.4	21	23.9	57	64.8	>0.05
	No	20	10.5	57	30.0	113	59.5	
Receive asthma information from relative	Yes	8	8.2	19	19.6	70	72.2	<0.05
	No	22	12.2	59	32.6	100	55.2	
Asthma history	Yes	16	11.6	45	32.6	77	55.8	>0.05
	No	14	10.0	33	23.6	93	66.4	
Asthma family history	Yes	11	10.4	29	27.4	66	62.3	>0.05
	No	19	11.0	49	28.5	104	60.5	
Severity of acute asthma attack	Mild	0	0	1	25.0	3	75.0	>0.05
	Moderate	25	11.8	58	27.5	128	60.7	
	Severe	5	7.9	19	30.2	39	61.9	
Severity of asthma	Intermittent	28	13.1	60	28.2	125	58.7	>0.05
	Mild persistent	1	2.1	14	29.2	33	68.8	
	Moderate persistent	1	5.9	4	23.5	12	70.6	
Caregiver's knowledge	Good	14	24.6	26	45.6	17	29.Ağu	<0.05
	Fair	15	11.5	38	29.0	78	59.5	
	Poor	1	1.1	14	15.6	75	83.3	
Caregiver's attitudes	Positive	21	20.2	38	36.5	45	43.3	<0.05
	Neutral	9	6.7	37	27.6	88	65.7	
	Negative	0	0	3	7.5	37	92.5	
Caregiver's practices	Good	26	20.8	49	39.2	50	40.0	<0.05
	Fair	3	3.4	19	21.3	67	75.3	
	Bad	1	1.6	10	15.6	53	82.8	

Table 3. Factors affecting caregiver' practices of using nebulizers and MDIs with spacers

		Practices of nebulizer					Practices of MDI with chamber				
		Good		Not good		p	Good		Not good		p
		n	%	n	%		n	%	n	%	
Gender of caregiver	Male	12	57.1	9	42.9	>0.05	3	14.3	18	85.7	>0.05
	Female	123	47.9	134	52.1		21	8.2	236	91.8	
Age of caregiver	<30	57	48.7	60	51.3	>0.05	10	8.5	107	91.5	>0.05
	31 - 40	64	50.4	63	49.6		10	7.9	117	92.1	
	>40	14	41.2	20	58.8		4	11.8	30	88.2	
Education of caregiver	Under high school	34	50.0	34	50.0	>0.05	3	4.4	65	95.6	>0.05
	High school	57	44.9	70	55.1		15	11.8	112	88.2	
	University and above	44	53.0	39	47.0		6	7.2	77	92.8	
Job of caregiver	Officer	42	54.5	35	45.5	>0.05	7	9.1	70	90.9	>0.05
	Worker	17	40.5	25	59.5		3	7.1	39	92.9	
	Farmer	6	46.2	7	53.8		0	0.0	13	100.0	
	Small business	30	50.8	29	49.2		5	8.5	54	91.5	
	Housewife	21	40.4	31	59.6		6	11.5	46	88.5	
	Other	19	54.3	16	45.7		3	8.6	32	91.4	
Asthma information from healthcare	Yes	102	49.8	103	50.2	>0.05	17	8.3	188	91.7	>0.05
	No	33	45.2	40	54.8		7	9.6	66	90.4	
Asthma information from books, newspapers	Yes	49	55.7	39	44.3	>0.05	11	12.5	77	87.5	>0.05
	No	86	45.3	104	54.7		13	6.8	177	93.2	
Asthma information from relatives	Yes	34	35.1	63	64.9	<0.05	5	5.2	92	94.8	>0.05
	No	101	55.8	80	44.2		19	10.5	162	89.5	
Patient's asthma history	Yes	67	48.6	71	51.4	>0.05	19	13.8	119	86.2	<0.05
	No	68	48.6	72	51.4		5	3.6	135	96.4	
Asthma family history	Yes	52	49.1	54	50.9	>0.05	10	9.4	96	90.6	>0.05
	No	83	48.3	89	51.7		14	8.1	158	91.9	
Severity of acute asthma attack	Mild	0	0.0	4	100.0	>0.05	0	0.0	4	100.0	>0.05
	Moderate	104	49.3	107	50.7		18	8.5	193	91.5	
	Severe	31	49.2	32	50.8		6	9.5	57	90.5	
Severity of asthma	Intermittent	107	50.2	106	49.8	>0.05	16	7.5	197	92.5	>0.05
	Mild persistent	20	41.7	28	58.3		4	8.3	44	91.7	
	Moderate persistent	8	47.1	9	52.9		4	23.5	13	76.5	
Caregiver's knowledge	Good	40	70.2	17	29.8	<0.05	9	15.8	48	84.2	>0.05
	Fair	66	50.4	65	49.6		10	7.6	121	92.4	
	Poor	29	32.2	61	67.8		5	5.6	85	94.4	
Caregiver's attitudes	Positive	54	51.9	50	48.1	>0.05	11	10.6	93	89.4	>0.05
	Neutral	68	50.7	66	49.3		12	9.0	122	91.0	
	Negative	13	32.5	27	67.5		1	2.5	39	97.5	
Caregiver's practices	Good	88	70.4	37	29.6	<0.05	11	8.8	114	91.2	>0.05
	Fair	30	33.7	59	66.3		7	7.9	82	92.1	
	Bad	17	26.6	47	73.4		6	9.4	58	90.6	
Caregiver's knowledge regarding the use reliever medication	Good	9	64.3	5	35.7	<0.05	3	21.4	11	78.6	>0.05
	Fair	25	75.8	8	24.2		5	15.2	28	84.8	
	Poor	101	43.7	130	56.3		16	6.9	215	93.1	
Caregiver's attitudes regarding the use reliever medication	Positive	17	65.4	9	34.6	<0.05	5	19.2	21	80.8	>0.05
	Neutral	48	61.5	30	38.5		7	9.0	71	91.0	
	Bad	70	40.2	104	59.8		12	6.9	162	93.1	
Caregiver's knowledge regarding the use controller medication	Good	9	69.2	4	30.8	>0.05	3	23.1	10	76.9	>0.05
	Fair	14	58.3	10	41.7		3	12.5	21	87.5	
	Poor	112	46.5	129	53.5		18	7.5	223	92.5	
Caregiver's attitudes regarding the use controller medication	Positive	23	65.7	12	34.3	<0.05	8	22.9	27	77.1	<0.05
	Neutral	29	55.8	23	44.2		6	11.5	46	88.5	
	Bad	83	43.5	108	56.5		10	5.2	181	94.8	

significantly associated with caregivers' gender, age, job, education, asthma information sources, asthma history of patients and their families, severity of flare-ups, and severity of asthma ($p > 0.05$) (Table 3).

The proportion of caregivers who had good practices of using MDIs with spacers in the group with a history of asthma was significantly higher than the group without a history of asthma (13.8% vs. 3.6%, $p < 0.05$).

Table 3 also shows that there was a significant relationship between attitudes toward the use of controller medications and practices of MDIs with spacers: 22.9% of the caregivers who had positive attitudes towards the use of controller medications had good practices toward the use of MDIs with spacers, whereas only 5.2% of those who had negative attitudes had positive practices ($p<0.05$).

Discussion

This study was the first research relating knowledge, attitudes and practices of caregivers towards using inhalers in Vietnamese children. So, there was not any data to compare with. The GINA and the asthma guidelines for the prevention and treatment highlight the value of education and the optimal use of inhaler devices [12]. In this study, the percentage of caregivers who had poor knowledge about general asthma medications, reliever medications and controller medications were very high: 83.5%, 83.1% and 86.7% respectively. Similar results were found in some other researches, such as in China, where results from an asthma-related KAPs survey showed that more than half the parents had a low level of knowledge [13]. In United Arab Emirates, the KAPs survey demonstrated that 52.6% of patients had poor knowledge [14]. In another study in Cairo, the result revealed that more than one-third of the mothers were found to have incomplete knowledge about asthma medications [15].

Our study found that caregivers' knowledge about asthma medication was not significantly related to caregivers' gender, age, job, education ($p > 0.05$). However, the proportion of caregivers with good knowledge about medications was significantly lower in the group that received asthma information from relatives compared to the group that did not receive those (0.0% vs. 7.7%, $p < 0.05$). These findings had some points which were similar to other studies and had some points which differ from some other studies. According to the study of Al-Ali et al., level of knowledge was not significantly associated with relation to child, marital status, or the age of the parents; but the percentage of parents who had good knowledge was significantly highest among parents of non-Emirati non-Arab background (16.1%), among those having educational level of university and above (16.6%) and among those being employed (13.6%) [14]. In the study conducted in Riyadh, Saudi Arabia, Al-Otaibi and Al-Ateeq explored that the majority of caregivers received their information about asthma from pediatricians (87%), followed by family physicians (44.3%), written materials (41.1%), and the Internet (40.7%); and suggested that for better control of asthma, more effort is needed to educate caregivers and to enhance their awareness about asthma, at both hospital and community levels [7].

Regarding attitudes, in this study, the percentage of caregivers who had a positive attitude toward the using of asthma medication, reliever asthma medication and controller asthma medication were 10.8%, 9.4% and 12.6%. These findings differed from those of other studies. According to Al-Ali et al., approximately half of participants (49%) had positive attitudes [14]. In the study of Zhao et al., most participants (89.95%) gave positive responses to ≥ 60% of the attitude questions [14]. Our study found that caregiver's attitudes about asthma medication were not significant associated with relation to child, caregivers' sex, job, asthma history of patients and their families, the severity of acute asthma attacks, asthma ($p> 0.05$). However, caregiver's attitudes about asthma medication were significant associated with caregivers' age, education ($p <0.05$). According to Al-Ali et al., attitudes toward the use of inhalers were associated with age of the parents, whereby elderly parents (>40) had more positive attitudes than younger parents (21-30 year-old) [14]. With regards to inhaler practices, the percentage of caregivers who have good practices in the use of nebulizer was 48.6%, whereas 51.4% had bad practices. There were only 8.6% of caregivers who had good practices in the use of MDIs with spacers and 91.4% of caregivers who had bad practices of that. In contrast with Al-Ali et al, the percentage of parents who had good practices in the use of inhaler was 56.2%, and more than two-thirds of participants who were given asthma action plans stated that they were confident about the use of inhaler [14]. According to Deis et al.' research, parents who were given a written asthma action plan for acute attacks were more confident in managing their child's asthma exacerbation, and they felt confident in using inhaler [16]. In our study, caregivers' practices of using nebulizers were not significant associated with relation to child, caregivers' sex, age, job and education, asthma history of patients and their families, the severity of acute asthma attack, asthma ($p > 0.05$). Our study differ from the result of Al-Ali et al which demonstrated that the relationship between education level and practices was significant associated [14]. However, our study showed that there was a significant relationship between caregivers' knowledge and attitudes about reliever medications, caregivers' attitudes about controller medications and their practice of using nebulizers ($p < 0.05$). And there was a significant relationship between attitudes toward the use of controller medications and practices of MDI with the chamber. These findings emphasize the importance of providing knowledge, explain more detail asthma disease for caregivers and guide them carefully on how to use inhalers.

Conclusion

Only a minority of the caregivers in this study had a good level of knowledge as well as positive attitudes regarding asthma, and the use of inhalers, and approximately half had good practices in the use of nebulizer and the minority of caregivers had good practices in the use of MDIs with spacers. Moreover, a good level of knowledge and positive attitudes were related to partial good practices. Besides, knowing patient's asthma history and receiving asthma information from relatives also play an important role in asthma practice. Education and training for caregivers reveal outstanding steps which contribute to treat asthma successfully, especially in practices of using inhaler. It is required that healthcare staff to educate and assess their understanding and practices.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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